In re Application of: VOELKEL

Serial No.: 09/744,016 Art Unit: 1652

Filed: May 7, 2001 Examiner: S. Swope

For: **METHOD FOR SCREENING OF MODULATORS OF CALCINEUIRN ACTIVITY**

ATTACHMENT B - AMENDMENTS TO CLAIMS

Please amend the claims as follows:

1. (Currently amended) Method for screening of modulators of calcineurin enzymatic activity, characterized in that an interaction between calcineurin and superoxide dismutase is monitored, comprising the following steps

- ~~forming~~ formation of a complex comprising at least calcineurin and superoxide dismutase ~~under incubation with~~ in the presence of at least one potential modulator,
- detecting the influence of the potential modulator by directly monitoring the complex formation and/or by monitoring the enzymatic activity~~, especially the enzymatical activity of the complex~~.

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2. (Original) Method according to claim 1, characterized in that the the superoxide dismutase is a Copper/Zinc-superoxide dismutase.

3. (Currently amended) Method according to claim 1, characterized in that ~~forming~~ formation of the complex is performed in the presence of the potential modulator.

4. (Previously amended) Method according to claim 1, characterized in that the potential modulator is added after the complex has been formed.

5. (Currently amended) Method according to claim 1, characterized in that the monitoring is performed by detection of labels~~, especially fluorescent labels~~.

6. (Currently amended) Method according to claim 1, characterized in that the calcineurin and/or the superoxide dismutase carry labels, ~~especially fluorescent markers,~~ wherein ~~preferably~~ the labels are enhanced green fluorescent protein.

7. (Currently amended) Method according to claim 6, characterized in that calcineurin and/or superoxide dismutase are expressed as fluorescent proteins~~, particularly as fusion proteins together with enhanced green fluorescent protein~~.

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8. (Previously amended) Method according to claim 1, characterized in that the monitoring of complex formation is performed by laser fluctuation correlation spectroscopy.

9. (Currently amended) Method according to claim 1, characterized in that calcineurin and superoxide dismutase are coexpressed in cells~~, especially in eukaryotic cells,~~ and that the complex formation is performed within the cell.

10. (Currently amended) Method according to claim 1, characterized in that calcineurin and/or superoxide dismutase are expressed in cells~~, especially in prokaryotic cells~~, and that calcineurin and/or superoxide dismutase are isolated and/or purified before the complex formation is performed.

11. (Currently amended) Method according to claim 10, characterized in that purification of calcineurin is achieved ~~by affinity chromatography, especially~~ by ferro-

nitrilotriacetat(NTA)-metal affinity chromatography.

12. (Currently amended) Method according to claim 10, characterized in that purification of superoxide dismutase is achieved ~~by affinity chromatography, especially~~ by copper/zinc-NTA-~~nitrilotriacetat~~-metal affinity chromatography.

13. (Previously amended) Method according to claim 1, characterized in that in the complex formation step, calmodulin and/or calcium are present ~~additionally calmodulin and/or calcium is added~~.

14. (Currently amended) Method according to claim 1, characterized in that the monitoring of the ~~enzymatical~~ enzymatic activity is performed by analyzing the phosphatase activity of calcineurin.

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15. (Currently amended) Method according to claim 14, characterized in that the phosphatase activity is analyzed by the use of at least one substrate, which preferably carries a label~~, especially a fluorescent label~~.

16. (Currently) Method according to claim 15, characterized in that the substrate is a peptide~~, especially a peptide~~ characterized by the amino acid sequence

Asp - Leu - Asp - Val - Pro - Ile - Pro - Gly - Arg - Phe - Asp - Arg - Arg - Val - Ser - Val - Ala - Ala - Glu.

17. (Currently amended) Method according to claim 15, characterized in that the substrate is a peptide containing a residue~~, especially a serine residue,~~ labeled with ~~fluoresceine~~

fluorescein.

18. (Currently amended) Method according to claim 3, characterized in that ~~prior or after detecting the influence of the potential modulator on the complex formation and/or complex activity~~ the influence of the potential modulator on the complex activity~~, especially the enzymatical activity of calcineurin~~ is detected ~~seperately~~ separately from the influence of the potential modulator on the complex formation and or/complex activity.

19. (Currently amended)) Method for screening of modulators of calcineurin activity~~, especially according to claim 1,~~ comprising:

a) determining the interaction of a potential modulator with either calcineurin or superoxide dismutase as a partner,

b) taking a potential modulator showing interaction with calcineurin or superoxide dismutase according to step a),

c) determining the interaction of said modulator taken in step b), with the other partner, namely calcineurin or superoxide dismutase, respectively, and

d) identifying the potential modulator showing interaction also according to step c).

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20. (Currently) Method according to claim 19, characterized in that calcineurin and/or superoxide dismutase comprises at least one tag~~, especially a histidine tag~~.

21. (Previously amended) Method according to claim 19, characterized in that said superoxide dismutase is a Copper/Zinc-

superoxide dismutase.

22. (Currently amended) Method according to claim 19, characterized in that calcineurin and/or superoxide dismutase is attached to a solid matrix~~, especially a Ni-NTA, Fe-NTA and/or CuZn-NTA matrix~~.

23. (Withdrawn from consideration)